SAN TRIMER ASSOCIATION

1250 Connecticut Avenue, NW • Suite 700 • Washington, DC 20036
Phone: 202-419-1500 • Email: info@regnet.com

January 13, 2011

Dr. Lori White
NTP Designated Federal Officer
National Institute of Environmental Health Sciences
P.O. Box 12233
MD K2-03
Research Triangle Park, NC 27709
Via Email: whiteld@niehs.nih.gov

RE: Comments on Draft NTP Technical Report on SAN Trimer

Dear Dr. White:

Attached please find comments from the SAN Trimer Association (SANTA) on the draft NTP Technical Report on SAN Trimer, which is scheduled for review at the January 26, 2011 meeting of the NTP Technical Reports Peer Review Panel (75 Fed. Reg. 73085; November 29, 2010). SANTA consists of companies that have an interest in SAN Trimer, including The Dow Chemical Company and the Saudi Basic Industries Corporation (SABIC). Also attached, please find comments from Drs. Haseman and McConnell on the draft Report.

Please note that if additional information is received from NTP in response to our December 22, 2010 request, SANTA will likely submit a supplemental response. If you have any questions, please contact me at (202) 419-1500 or rfensterheim@regnet.com.

Sincerely,

[Redacted]

Robert J. Fensterheim
Executive Director

SAN TRIMER ASSOCIATION

1250 Connecticut Avenue, NW • Suite 700 • Washington, DC 20036
Phone: 202-419-1500 • Email: info@regnet.com

Comments on the National Toxicology Program Technical Report on SAN Trimer (TR 573) Submitted For Consideration by the NTP Technical Reports Peer Review Panel (75 Fed. Reg. 73085; November 29, 2010)

January 13, 2011

I. Introduction and Summary

The SAN Trimer Association (also referred to as SANTA) is pleased to submit comments on the draft NTP Technical Report on SAN Trimer (hereinafter "Draft TR 573"), which is scheduled for review at the January 26, 2011 meeting of the NTP Technical Reports Peer Review Panel (75 Fed. Reg. 73085; November 29, 2010).

As noted in SANTA's letter of December 22, 2010 (attached), comprehensively reviewing Draft TR 573 has been somewhat impeded by the lack of some of the underlying data and other information that are directly relevant to the overall conclusions/evaluation presented by NTP. For this reason, SANTA submitted a formal request for this supplemental information including the:

- Report of the Pathology Working Group and the Special Emphasis Panel. According to the Draft TR 573, there were two rounds of histopathology for the nervous system: an initial and expanded review. The methodology used in the evaluation of the brain and spinal cords for both the initial and expanded histopathology reviews are important to consider in assessing the appropriateness of the report's conclusions.

- Individual animal pathology data for the extended evaluation, particularly relating to the spinal cord, including the approximate amount of spinal nerve root tissue examined from the different animals, e.g., the number of nerve fibers examined from each animal.

- Statistical analysis supporting the draft report conclusions, particularly those described on Table 19.

- Guidance relating to evaluation of nerve degeneration severity, e.g., was the severity of nerve degeneration recorded as an average of the nerves examined or the worst case?

- Genetic toxicology study reports for the Ames assay, Comet assay and Micronucleus assay, including underlying information such as relevant historical control and histopathology data.

The December 22 letter further requested an additional 30 days of time following receipt of the supplemental information in order to review and develop comments on any of the additional information provided. This supplemental information should be made available to all stakeholders, including the Peer Review Panel, given that the information may impact the interpretation and conclusions presented in the Draft NTP Technical report.

As discussed in these comments, the SAN Trimer Association believes that the Draft TR 573 presents conclusions and viewpoints that are not supported by the underlying data NTP generated as part of the chronic bioassay and other studies conducted on SAN Trimer. Of particular concern is the draft's conclusion that SAN Trimer presents "Equivocal Evidence" of carcinogenic activity in male rats. Rather, for reasons described below, in our view the data support a conclusion of "No Evidence" of carcinogenic activity. Similarly, the report's assertion that SAN Trimer induced peripheral nerve degeneration is questionable as these observations may be likely the result of age-related neurodegeneration. While the Draft TR 573 presents a statistically significant poly-3 for nerve degeneration, it is unclear how these statistics were derived and whether they support a contention of adverse effects from the test compound, particularly given questions about the appropriateness of the standard poly-3 test.

Other issues discussed in these comments that should be considered by NTP include:

- SAN Trimer is not mutagenic in bacterial or mammalian cells, and the DNA damage reported needs to be considered in context with the cytotoxicity and other underlying information that has not yet been provided.

- The bone marrow hyperplasia is most likely a normal adaptive response to regenerative proliferation from hepatotoxicity.

Lastly, it appears that the Draft TR 573 has tended to infer effects on SAN Trimer, partially because of issues associated with acrylonitrile, one of the starting monomers in the manufacture of polymers affiliated with SAN Trimer. While cancer bioassays of acrylonitrile have identified an association with astrocytomas in exposed rodents, given that no acrylonitrile was found in the test compound and the incidence of astrocytomas found with SAN Trimer was exceedingly low, there is no justification to infer acrylonitrile's rodent carcinogenic profile when evaluating SAN Trimer.

II. There Is No Clear Justification for the Report's Conclusion that SAN Trimer Present "Equivocal Evidence" of Carcinogenic Activity

The draft Report states that:

> Under the conditions of this 2-year feed study preceded by perinatal exposure, there was *equivocal evidence of carcinogenic activity* of SAN Trimer in male F344/N rats based on the occurrence of astrocytomas and granular cell tumors in the brain and spinal cord.

According to NTP, an "equivocal evidence" classification is assigned to "studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related." As discussed in greater detail in the attached review by Dr. Joe Haseman and by Dr. Gene McConnell, there is inadequate justification to classify the study as providing "equivocal evidence," as there is inadequate basis to suggest that there is a compound related increase in the incidence of astrocytomas and granular cell tumors in the brain and spinal cord. Rather, the data are much more consistent with a "No Evidence" classification. SANTA arrives at this position based on the following:

- The incidences of brain tumors observed in all of the dosed groups were within the historical control range. Specifically, the 4% incidence of astrocytomas found in male rats in the high dose group is within the historical control incidence reported for this tumor type. Additionally, the Sills *et al*. (1999) paper cites a 4% control rate of granular cell tumors of

the brain in male F344 rats, which exceeds the incidence of granular cell tumors (2%) observed in any SAN Trimer male rat group in the NTP study.

- The attached review by Haseman identifies five previous NTP studies (CI Pigment Red 23; 4-Methylimidazole, primidone, tetracycline HCL, and eugenol), with brain tumor occurrences in male rats very similar to that seen for SAN Trimer (in fact, one had identical tumor incidences), and all five were judged by the NTP to provide "No evidence" of carcinogenic activity.

- This view is reinforced by the fact that only one additional spinal cord tumor (in the low dose group) was identified following the extensive additional histopathology evaluation conducted of the nervous tissues, which included the spinal cords for all 200 male rats and nine additional sections of brain for each animal, resulting in 1800 additional brain sections. As noted by Haseman, "If this brain/spinal cord tumor effect had been real (or even "equivocal"), it is probable that this additional histopathology evaluation would have found additional brain or spinal cord tumors in the mid- or high-dose group. Clearly, the extended histopathology evaluation supports a "No Evidence" call for male rats."

- Increased nerve degeneration in the higher dose group is more likely the result of the improved survival associated with exposure to the test compound. It is well established that spontaneous degeneration within the peripheral nerves is found with increased severity in older-aged rats. As such, the longer survival times for the test article-treated male groups, as compared to the concurrent control group, may itself be associated with the slight, non-statistically significant increase observed – this should not be considered a direct compound-related effect. While we recognize that the poly-3 test is intended to account for differential longevity, the standard poly-3 test may not be appropriate for evaluating nerve fiber degeneration in this study for a variety of reasons as discussed in Haseman's report.

It is further significant to note that the results from the poly-3 statistical test for the astrocytomas and other nervous tissues were not reported on Table A2 of the Draft TR 573 along with the other anatomical sites/tissues evaluated. This is somewhat surprising given that the astrocytomas and granular cell tumors formed the basis for the proposed classification. When revising the report, NTP should present explicitly the results of the poly-3 analyses of brain tumors that led to the "equivocal evidence" call, as well as discuss the possible impact of improved survival on the test results. Also, NTP should more fully discuss the increased survival associated with the test compound as well as the

statistical evaluation intended to account for the larger number of animals that survived to the end of the study in contrast with the number of animals in the control group. We note that only 36 of the 50 animals survived to the end of the study in the male rat control group, in contrast with the 44 animals surviving in the high dose group.

III. There Is Insufficient Evidence to Support the Draft Report's Assertion That Peripheral Nerve Degeneration was Treatment-Related

The conclusion section of the draft NTP Report states:

> Peripheral nerve degeneration and nonneoplastic lesions of the bone marrow and liver in male and female F344/N rats and urinary bladder lesions in female F344/N rats were attributed to exposure to SAN Trimer.

The Draft TR "Abstract" goes on to highlight this nerve degeneration finding, specifically emphasizing an increasing trend in the severity of the degeneration with increasing exposure:

> There were statistically significant increases in the incidence of spinal nerve root degeneration in 1,600 ppm males and the incidences of sciatic nerve degeneration in 800 and 1,600 ppm females. More importantly, there were increases in the severities of both nerve lesions in males and in the severity of spinal nerve root degeneration in females.

The conclusion regarding nerve degeneration appears to have partially contributed to the draft report's proposed classification that the SAN Trimer study suggests equivocal evidence of carcinogenic activity. For reasons given in the attached reports by Drs. Haseman and McConnell, SANTA believes that the Draft's emphasis on compound-related nerve degeneration as supporting evidence for the cancer classification should be reconsidered. Moreover, attaching some importance to a compound-related increase in nerve fiber degeneration severity seems misplaced based on severity scores ranging from 1.0 to 1.3, as these differences in grades are likely not biologically significant. This is particularly the case given that mild degrees of spinal nerve fiber degeneration are often seen in older-age rats and in this particular study, there was an unusually high survival in the high- and mid-dose groups. As already noted, at the end of the study there were

44/50 male rats surviving in the high dose group and 39/50 in the low and mid-dose groups as compared with only 36/50 animals in the control group.

As NTP reviews the issues associated with nerve degeneration, consideration should be given to the issues raised by Haseman regarding the appropriateness of using the standard poly-3 test and further discuss how severity was graded in situations where multiple spinal nerve roots and/or sciatic nerves for each animal were evaluated.

Both Haseman and McConnell raised questions regarding the manner in which the nerve fibers were graded for degeneration and whether the scoring was done in a "blinded" fashion, or if the reviewing pathologists were aware of the dose group associated with the particular nerve tissue.

IV. The Reported DNA Damage from Exposure to SAN Trimer is of Questionable Significance Based on the Information Available

The Draft TR notes that the genetic toxicology studies conducted by NTP confirmed the lack of mutagenicity reported in the previous bacterial and mammalian studies. However, NTP reports that SAN Trimer was associated with increased levels of DNA damage in brain cells, measured by the comet assay and increased chromosomal damage in peripheral blood reticulocytes, measured by the micronucleus assay. The report hypothesizes that these positive results "may be cause for concern" (Draft TR, page 94).

To evaluate the significance of these genetox studies in the context of carcinogenesis, it is important to recognize at the outset that the assessment of tumorogensis following lifetime exposure to SAN Trimer did not result in a statistically significant increase in tumors. Thus, there is no technical foundation to support a correlation between the purported DNA damage and cancer. At the same time, the ability to fully review these genotoxicity studies is complicated by the lack of details regarding their conduct. As already noted, the full reports for these studies have not been made available despite the

request by the SAN Trimer Association. Without the full reports, it is not feasible to fully assess their relevance.

In general, the comet assay does not provide direct evidence of DNA mutations; the assay is, however, an extremely sensitive means to measure DNA damage. There are numerous issues associated with the conduct of the assay that complicate its interpretation. The fact that there is no standard protocol that has been accepted further impacts the ability to rely on this assay as a predictor of carcinogenesis. The assay is generally considered a much more useful tool for purposes of evaluating modes of action rather than as a predictor of carcinogenesis. (Several of the key issues associated with the use of the comet assay are presented in comments on EPA Document: Framework for Determining a Mutagenic Mode of Action for Carcinogenicity developed by Albertini and Walker (2007).)

As described in comments submitted to NTP by the Dow Chemical Company[1] in greater detail, the lack of available information on cytotoxicity in tissues complicates interpreting the results from the gene tox studies given that the increased damage observed may be an effect of tissue toxicity rather than a direct effect from exposure to SAN Trimer. This concern is not hypothetical but likely given the nearly lethal dose of SAN Trimer that the animals received.

Since the report does not provide clinical observation data for the exposed animals and no histopathology assessments of the tissues used for the comet assays are presented, it is not possible to effectively interpret the reported observations. As also discussed in greater detail in the Dow comments, the lack of DNA damage in the rats given 75 mkd, a dose that is very close to the top dose in the cancer bioassay (1600 ppm ~80 mkd), suggests that there should be no concern for mutation effects.

It is further worth noting that the response reported was minimal in comparison to the very high exposure at the top dose. Nonetheless, it is striking that the level of

[1] The Dow Chemical Company is a member of SANTA and has submitted separate more detailed comments on issues associated with the genetic toxicology studies conducted on SAN Trimer.

significance in response is greater in females than males even though there were no brain tumors observed in females.

Similar issues exist for the micronucleus assay. The lack of a complete report for this assay complicates its interpretation. There is particular interest in reviewing the clinical observation data and specifically the body temperature data for the individual animals. The relevance of these findings can only be assessed following review of the underlying data including the historical control range for this testing facility.

V. The Reported Bone Marrow Hyperplasia is of Questionable Relevance

The draft NTP technical report highlights a statistically significant increased in incidences of bone marrow hyperplasia and bone marrow granulomatous inflammation as biologically significant given that these lesions are "very rare." There are numerous issues associated with these results that do not appear to have been adequately discussed. There is no discussion over whether the bone marrow hyperplasia is an adaptive response to the hepatic toxicity with regenerative proliferation-induced stimulation of hematopoietic stem cell to replace the loss of hepatic cells. The increased hyperplasia also appears to have little consequence to the animals given that exposure to SAN Trimer reduced the incidence of leukemia at all doses with a monotonic dose-response. It is further curious to note that the severity grade for the hyperplasia was greatest in the control animals over the treated even when compared against the top dose, which had the highest number of animals with hyperplasia.

VI. Conclusion

SAN Trimer did not produce a statistically significant increase in any tumor type as reflected in the Draft TR 573. Moreover, the very few tumors that were reported do not rise to a classification of "Equivocal Evidence" based on the small numbers observed and the extensive sectioning of the brains and spinal cord from the exposed animals. Other compounds reviewed by NTP with similar brain tumor response were considered as "No

Evidence" of Carcinogenic Activity and a similar classification should be assigned to SAN Trimer. Furthermore, the slight increases in the incidence and severity of nerve fiber degeneration of the sciatic nerve and spinal nerve roots are not statistically or biologically significant in male rats. Instead, these marginal effects are likely related to the improved survival observed in the top dose group relative to controls and possibly the lack of blinding of the extended evaluation of brain and spinal cord.

SAN TRIMER ASSOCIATION

1250 Connecticut Avenue, NW • Suite 700 • Washington, DC 20036
Phone: 202-419-1500 • Email: info@regnet.com

December 22, 2010

Dr. Lori White
NTP Designated Federal Officer
National Institute of Environmental Health Sciences
P.O. Box 12233
MD K2–03
Research Triangle Park, NC 27709
whiteld@niehs.nih.gov

Dear Dr White:

On behalf of the SAN Trimer Association (hereinafter SANTA), I am writing to request supplemental information relating to the draft NTP Technical Report (TR) on SAN Trimer, which is scheduled for review at the January 26, 2011 meeting of the NTP Technical Reports Peer Review Panel. According to the November 29, 2010, Federal Register notice (75 Fed. Reg. 73085), comments on the report are due by January 12, 2011.

The members of SANTA and their pathology/statistical experts, have been reviewing the draft SAN Trimer NTP Technical Report and concluded that in order to effectively comment on the report, it is necessary to receive supplemental information – information which we feel is critical to provide a greater understanding of the underlying basis for the conclusions and findings described in the report. We ask that NTP provide this information, and any other information relevant to these topics, as soon as possible. We further request that NTP grant additional time to comment on the report; an additional 30 days following receipt of the requested information should be sufficient to complete our review and to submit formal comments. The following information is requested:

1. Special Emphasis Panel Report – We would like to receive a copy of the full Special Emphasis Panel Report. Page 16 of the SAN TR states that a "summary of the Special Emphasis Panel's remarks will appear in a future draft of this report." We feel it is important to review this report to prepare comments.

2. Additional Details Regarding the Initial and Expanded Histopathology Review - According to the draft TR, there were two rounds of histopathology for the nervous system: an initial and expanded review of the central and peripheral nervous systems. We would like to receive the reports of these reviews, including reports of any Pathology Working Group (PWG) meetings.

    - We are particularly interested in the details of the results of these reviews as well as the methodology employed during the pathology reviews. For example, were additional sections taken from the wet tissue in addition to the paraffin blocks for the expanded review? Was the slide review conducted in a "blind" fashion regarding dose levels or did the reviewing pathologist know which tissues were associated with the different dose levels?

- We would also like to receive the individual animal pathology data for the extended evaluation, particularly relating to the spinal cord. We are specifically interested in understanding, which animals had nerve fiber degeneration, the corresponding severity, the approximate amount of spinal nerve root tissue examined from the different animals, e.g., the number and approximate lengths of nerve fibers examined from each animal.

3. Statistical Analysis Supporting the TR Conclusions - We are particularly interested in a copy of the poly-3 statistical evaluation of nerve fiber degeneration, which underlie the reported "p" values that are found on Table 19, as this appears to be the basis for the conclusion of statistical significance.

   - Any guidance or other information that is germane to evaluating nerve degeneration severity. The draft TR draws a distinction between a 1.0 severity rating and a 1.3, suggesting that this difference is biologically meaningful. We would appreciate receiving any guidance that explains the significance of this difference as well as examples of other TRs where a similar position was taken.

4. Genetic Toxicology Studies and Underlying Information- The draft report briefly describes the results from a few genetic toxicology studies noting that there were "positive comet assay results" and a study showing a "significant increases in the frequencies of micronucleated reticulocytes" in peripheral blood of male and female juvenile rats. With regards to these studies, we would appreciate receiving:

   - The study reports for the Ames assay, Comet assay and Micronucleus assay, which we assume would contain the statistical evaluation; if not, please provide the statistical reports as well;
   - Historical control data for the comet and micronucleus assays for corn oil vehicle control and for positive controls; and,
   - Histopathology data as well as cytotoxicity information from the tissues evaluated in Comet assay.

* * *

As already noted, we appreciate your assistance in gathering and providing the requested information and hope that NTP will look favorably on the additional 30 days of time requested to prepare and submit comments. While recognizing that we are in the midst of the holiday season, I will plan to call your office to confirm receipt of this request and to address any questions.

Sincerely,

[Redacted]

Robert J. Fensterheim, MPH
Executive Director, SAN Trimer Association